potassium (BK_{Ca}) channels and inducing membrane hyperpolarization and vasorelaxation.
In Fluo-4-AM-loaded mesenteric myocytes, application of the Epac-specific cAMP analogue 8-pCPT-2'-*O*-Me-cAMP-AM (10μM) increased spark frequency from 0.045 ± 0.008 sparks/s/μm under basal conditions to 0.103 ± 0.022 sparks/s/μm ($p<0.05$). Importantly this increase also occurred in the presence of myristoylated PKI amide (14-22), a potent and selective inhibitor of PKA.
Application of 8-pCPT-2'-*O*-Me-cAMP-AM (5μM) reversibly increased both the frequency (0.94 ± 0.25 to 2.30 ± 0.72 s^{-1}) and amplitude (23.9 ± 3.3 to 35.8 ± 7.7 pA) of spontaneous transient outward currents (STOCs) recorded in isolated mesenteric myocytes ($n=7$; $p<0.05$). These currents were sensitive to the selective BK_{Ca} channel blocker, iberiotoxin (100nM), and to ryanodine (30μM). In addition, current clamp recordings of isolated myocytes showed a 7.43 ± 0.96 mV ($n=4$) hyperpolarization in response to exposure to 8-pCPT-2'-*O*-Me-cAMP-AM (5μM).
Our data suggest a novel cAMP-dependent mechanism in mesenteric smooth muscle cells whereby activation of Epac facilitates localized Ca^{2+} release which activates surface BK_{Ca} channels to modulate membrane potential and vascular tone.
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3163-Pos Board B318
Methods to Measure and Analyze Ciliary Beat Activity: Calcium Influx-Mediated Cilia Mechanosensitivity
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Airway ciliary beat activity (CBA) plays a pivotal role in protecting the body by removing mucus and pathogens from the respiratory tract. Since CBA is complicated and can not be characterized by merely frequency, we recorded CBA using laser confocal line scanning and defined 6 parameters for describing CBA. The values of these parameters were all above 0 when measured in beating ciliated cells from mouse tracheae. We subsequently used 10 μM adenosine-5′-triphosphate (ATP) to stimulate ciliated cells and simultaneously recorded intracellular Ca^{2+} levels and CBA. We found that intracellular Ca^{2+} levels first increased, followed by an increase in CBA. Among the 6 parameters, frequency, amplitude, and integrated area significantly increased, whereas rise time, decay time, and full duration at half maximum markedly decreased. The results suggest that these 6 parameters are appropriate for assessing CBA and that increased intracellular Ca^{2+} levels might enhance CBA. We next used our established methods to observe changes in mechanically stimulated cilia tips. We found that mechanical stimulation-induced changes in both intracellular Ca^{2+} levels and CBA were not only similar to those induced by ATP but were also blocked by treatment with a Ca^{2+} chelator, BAPTA-AM (10 μM) for 10 min. Moreover, while the same blockage were observed under Ca^{2+}-free conditions, addition of 2 mM Ca^{2+} into the chamber restored increases in both intracellular Ca^{2+} levels and CBA. Taken together, we have provided a novel method for real time measurement and complete analysis of CBA as well as demonstrated that mechanical stimulation of cilia tips resulted in Ca^{2+} influx that led to increased intracellular Ca^{2+} levels, which in turn triggered CBA enhancement.

3164-Pos Board B319
Spatial Distribution Patterns of Alpha-CaMKII in Hippocampal Tissue Suggest Role for Transient Not Persistent Translocation in Chemical LTP and Persistent Clustering in Ischemia
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Calcium.calmodulin-dependent protein kinase II (alpha-CaMKII) forms punctate structures in dendritic spines and in the soma of hippocampal pyramidal neurons upon calcium elevation evoked by N-methyl-D-aspartic acid receptor (NMDAR) stimulation. Though advocated to be relevant to memory formation, it is not clear whether spinal accumulation represents translocation to and interaction with NMDAR or, as somal punctate accumulation, it is the result of self-association representing clustering of alpha-CaMKII. Alpha-CaMKII punctate accumulation can be transient or persistent, the significance of temporal pattern to memory formation and excito-toxicity is however not clear either. In order to obtain a clearer understanding of the state of alpha-CaMKII in memory formation and excito-toxic insult, e.g. ischemia, we carried out an extensive ultrastructural study of alpha-CaMKII distribution in control, in chemically induced long-term potentiation (cLTP) and ischemic conditions in rat CA1 hippocampal slices by electron microscopy and immunogold labeling. We found preferential localization of alpha-CaMKII to post-synaptic densities in all three conditions but no significant differences between the distributions of alpha-CaMKII, phospho-Thr286-alpha-CaMKII and phospho-Thr305-alpha-CaMKII when comparing control and cLTP treated slices. The number of alpha-CaMKII clusters was significantly greater in the ischemic than in the control and cLTP treated slices. Thus, contrary to expectations, we found no evidence of significant persistent alpha-CaMKII accumulation in the dendritic spines in cLTP and conclude that transient rather than persistent translocation of alpha-CaMKII to the post-synaptic membrane is relevant to LTP induction. This is consistent with a switch-like rather than a resident role for alpha-CaMKII in memory formation. Our data further show that cluster formation is a hallmark of ischemic insult and not relevant to memory formation.

3165-Pos Board B320
Orai-Stim Mediated Ca^{2+} Release from Secretory Granules Revealed by a Novel Ca^{2+} and pH Probe
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Secretory granules (SGs) bud from the trans-Golgi network and during subsequent maturation, acquire an acidic intraluminal pH, electron-dense cores, and matrices that bind Ca^{2+} and other ions. Total Ca^{2+} in SGs is remarkably high, 30-40 mM. The free Ca^{2+} is significantly lower, with estimates ranging from 10-80 μM. Understanding roles for this calcium and potential mechanisms of release is hampered by the difficulty in measuring SG calcium directly in living cells. We adapted the FRET-based D1-ER probe to develop a novel probe (D1-SG) to measure calcium and pH in secretory granules. It significantly localizes to SGs and reports resting free Ca^{2+} of 69 ± 15 μM and pH 5.8. Application of ATP resulted in a slow monotonic decrease in SG Ca^{2+} temporally correlated with the occurrence of store-operated calcium-entry (SOCE). Further investigation revealed a novel receptor-mediated mechanism of calcium release from SGs that involves store-operated channels. SG Ca^{2+} release is completely antagonized by a SOCE antagonist, by switching to Ca^{2+}-free medium, and by overexpression of a dominant-negative Orai1(E106A). Overexpression of the CRAC activation domain (CAD) of STIM1 resulted in a decrease of resting SG Ca^{2+} by ~75% and completely abolished the ATP-mediated release of Ca^{2+} from SG's. Overexpression of a dominant negative CAD construct (CAD-A376K) induced no significant changes in SG Ca^{2+}. Colocalization analysis suggests that, like the plasma membrane, SG membranes also possess Orai1 channels, and during SG Ca^{2+} release, colocalization between SGs and STIM1 increases. We propose Orai channel opening on SGs membranes as a potential new mode of calcium release from SGs that may serve to raise local cytoplasmic calcium concentrations and aid in refilling intracellular calcium stores of the endoplasmic reticulum and exocytosis. (Grants NS08174/GM83913/F32 DC068982/K01 MH086119/NSFChina/985DEChina).

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Structural Requirements of N- and C-Terminal Orai Strands to allow Maximal Store-Operated Ca^{2+} Current Activation by STIM1
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The Ca^{2+} sensor protein STIM1 anchored in the ER membrane and the pore-forming Orai protein in the plasma membrane represent the key components of Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Recent publications have demonstrated that CRAC current activation requires 8 STIM1 molecules together with an Orai complex formed by 4 subunits. Channel gating necessitates direct binding of the cytosolic portion of STIM1 with both N- and C-termini of the Orai channel. A single point mutation L273S in Orai1 C-terminus abolishes the interaction with STIM1 and followingly the activation of Orai1 currents. A truncation till to the fourth amino acid of the N-terminal conserved region (aa73-89) results in the loss of STIM1 binding to the N-terminus and STIM1-mediated activation. We employed Orai1 dimers to identify whether all N-/C-terminal domains within an Orai concatamer are required to be intact for maximal Ca^{2+} current activation. Orai dimers with one wild-type and one L273S-mutant subunit displayed a similar extent of maximal currents like and an unchanged inactivation compared to wild-type proteins. These results suggest that either less than 8 STIM1 are even sufficient to activate Orai1 channels maximally or the L273S mutation is not sufficient to impair the recruitment of eight STIM1 proteins to an Orai1 oligomer containing

wild-type and mutant C-termini with a 1:1 stoichiometry. Orai dimers containing one inactive N-truncation mutant and one wild-type form showed preserved but about 40% significantly reduced Orai1 currents. Hence, the lack of half of functional N-termini within an Orai channel already reduces the current extent. Interestingly, N-termini seem to contribute in a more dominant manner to the generation of maximal Ca^{2+} currents compared to Orai1 C-termini. (supported by T466 to I.D. and P22565 to C.R.)

3167-Pos Board B322

Increased Microtubule Network Stability Underlies X-ROS in Dystrophic Skeletal Muscle

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In dystrophic muscle, an increase in reactive oxygen species (ROS) production and sarcolemmal calcium (Ca^{2+}) influx contributes to stretch-induced muscle damage however mechanistic insights into the activation of these pathways is lacking. In *mdx* myofibers (murine Duchenne muscular dystrophy), we have demonstrated that with mechanical stretch, the microtubule (MT) cytoskeleton is a critical mechano-transduction element for the activation of NADPH oxidase2 (Nox2) derived ROS production; a pathway we term X-ROS signaling [1]. Downstream, we showed that X-ROS sensitized stretch activated channels (SACs) to increase sarcolemmal Ca^{2+} influx during stretch. The significance of the MT cytoskeleton activation of X-ROS in *mdx* was revealed when the acute targeting of MT density proffered protection from contraction induced damage. In mammalian cells, the MT network is a dynamic structure in which MT density is determined by the stability of MT filaments. Our initial studies used acute pharmacological stabilization (taxol) or destabilization (colchicine) to establish MT network density as critical for the mechano-activation of X-ROS. We now interrogate critical upstream pathways and use new pharmacological and molecular approaches to explore the role of endogenous modulators of MT stability and how they may contribute to the enhanced X-ROS in dystrophic skeletal muscle.

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3168-Pos Board B323

MCUR1 is an Essential Component of Mitochondrial Ca^{2+} Uptake that regulates Cellular Metabolism

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Ca^{2+} flux across the mitochondrial inner membrane regulates bioenergetics, cytoplasmic Ca^{2+} signals and activation of cell death pathways. Mitochondrial Ca^{2+} uptake occurs at regions of close apposition with intracellular Ca^{2+} release sites, driven by the inner membrane voltage generated by oxidative phosphorylation and mediated by a Ca^{2+} selective ion channel (MiCa) called the uniporter whose complete molecular identity remains unknown. Mitochondrial calcium uniporter (MCU) was recently identified as the likely ion-conducting pore. In addition, MICU1 was identified as a mitochondrial regulator of uniporter-mediated Ca^{2+} uptake in HeLa cells. Here we identified CCDC90A, hereafter referred to as MCUR1 (Mitochondrial Calcium Uniporter Regulator 1), an integral membrane protein required for MCU-dependent mitochondrial Ca^{2+} uptake. MCUR1 binds to MCU and regulates ruthenium red-sensitive MCU-dependent Ca^{2+} uptake. MCUR1 knockdown does not alter MCU localization, but abrogates Ca^{2+} uptake by energized mitochondria in intact and permeabilized cells. Ablation of MCUR1 disrupts oxidative phosphorylation, lowers cellular ATP, and activates AMP kinase-dependent pro-survival autophagy. Thus, MCUR1 is a critical component of a mitochondrial uniporter channel complex required for mitochondrial Ca^{2+} uptake and maintenance of normal cellular bioenergetics.

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A New Approach to Evaluation of Mitochondrial Ca-Signaling in Cardiomyocytes

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Cardiac EC-coupling is mainly controlled by I_{ca}-gated Ca-release, but the extent to which mitochondria contribute to the overall Ca-signaling remains controversial. To examine the possible role of mitochondria in Ca-signaling, we used a lower Ca-affinity mitochondrial probe, mitycamE31Q (300MOI, 48-72h) in conjunction with TMRE and Fura-2AM in cultured neonatal rat and adult feline cardiomyocytes using a dual wavelength TIRF imaging system (< 150nm penetration, 10Hz). Calcium was simultaneously measured in cytosol and mitochondria using mitycamE31Q and Fura-2. TMRE was used to monitor mitochondrial membrane potential. MitycamE31Q and TMRE staining of feline cardiomyocytes showed fluorescent longitudinal bands. Caffeine application caused a rapid decline in Fura-2 signal (increased Ca^{2+}) that recovered slowly while mitycamE31Q signal initially increased (loss of $[Ca^{2+}]_m$ or $[H^+]_m$) before decaying back to or below baseline ($[Ca^{2+}]_m$ or $[H^+]_m$ uptake). Simultaneously measured TMRE signal showed mitochondrial depolarization and repolarization. Low Na^+ slowly decreases both Fura-2AM and mitycamE31Q signals, consistent with rise of cytosolic and mitochondrial Ca^{2+}. FCCP produced little changes in the Fura-2AM signal, but increased the rate of spontaneous Ca-waves as it increased mitochondrial Ca^{2+} and depolarized mitochondrial membrane potential (increase TMRE signal). In contrast, single mitochondrial signals demonstrated heterogeneous populations of mitochondrial activity. Our findings suggest that Ca^{2+} in the mitochondria not only shows transients that are generally delayed and attenuated compared to the cytosolic Ca^{2+} signals, but at intervals can also be released locally and spontaneously. When synchronized, the local mitochondrial Ca^{2+} releases generate large cellular cytosolic Ca^{2+} releases that may interfere with normal Ca^{2+} signaling. (Support: NIH, RO1-HL16152, RO1-HL107600).

3170-Pos Board B325

Bidirectional Coupling between Ryanodine Receptors and Store-Operated Calcium Entry in Human T Cells

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Store-operated Ca^{2+} entry (SOCE) via plasmalemmal Ca^{2+} release-activated Ca^{2+} (CRAC) channels activated in response to Ca^{2+} store depletion is a major source for $[Ca^{2+}]_i$ elevation in T cells. Having the tools formanipulation of T cell SOCE would be beneficial for modifying immune responses in humans. We investigated the expression and function of ryanodine receptors (RyR) in primary human T cells. Transcriptsof all three RyR isoforms *(RyR1, RyR2*, and *RyR3)* were detected in human T cells with *RyR1* appearing to be the most abundant. *RyR1/2* transcript levels increased, whereas those of *RyR3* decreased after T cell activation. RyR1/2 protein immunoreactivity was detected in activated, but not in resting T cells. The RyR agonist caffeine evoked Ca^{2+} release from the store in activated T cells but not in resting T cells, indicating that RyR are functionally upregulated in activated T cells compared with resting T cells. In the presence of SOCE via CRAC channels, RyR blockers reduced the Ca^{2+} leak from the endoplasmic reticulum (ER) and the magnitude of SOCE, indicating that a positive feedback relationship exists between RyR and CRAC channels. Overexpression of fluorescently tagged RyR2 and stromal interaction molecule 1 (STIM1), an ER Ca^{2+} sensor gating CRAC channels, in HEK293 cells revealed that RyR are co-localized with STIM1 in the puncta formed after store depletion. We conclude that in primary human T cells, the RyR are coupled to CRAC channel machinery such that SOCE activates RyR via a Ca^{2+}-induced Ca^{2+} release mechanism, which in turn reduces the Ca^{2+} concentration within the ER lumen in the vicinity of STIM1, thus facilitating SOCE by reducing store-dependent CRAC channel inactivation. Treatment with RyR blockers suppressed activated T cell expansion, demonstrating the functional importance of RyR in T cells.

3171-Pos Board B326

Shock Waves Simulating Blast-Induced Traumatic Brain Injury Activate Purinergic Signaling in Astrocytes

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While progress has been made in physically protecting individuals from experiencing bTBI, there are no countermeasures or drug treatments to ameliorate the effects of bTBI after exposure. Historically bTBI research focused on neuronal protection and the effect of neuronal trauma with less attention to the effect of injury to astrocytes. We have developed a pneumatic device that delivers shockwaves, similar to those known to induce bTBI, within a chamber optimal for fluorescence microscopy. Abrupt changes in pressure can be created with and without the presence of shear forces at the surface of cells. We discovered that exposure of primary cultures of human central nervous